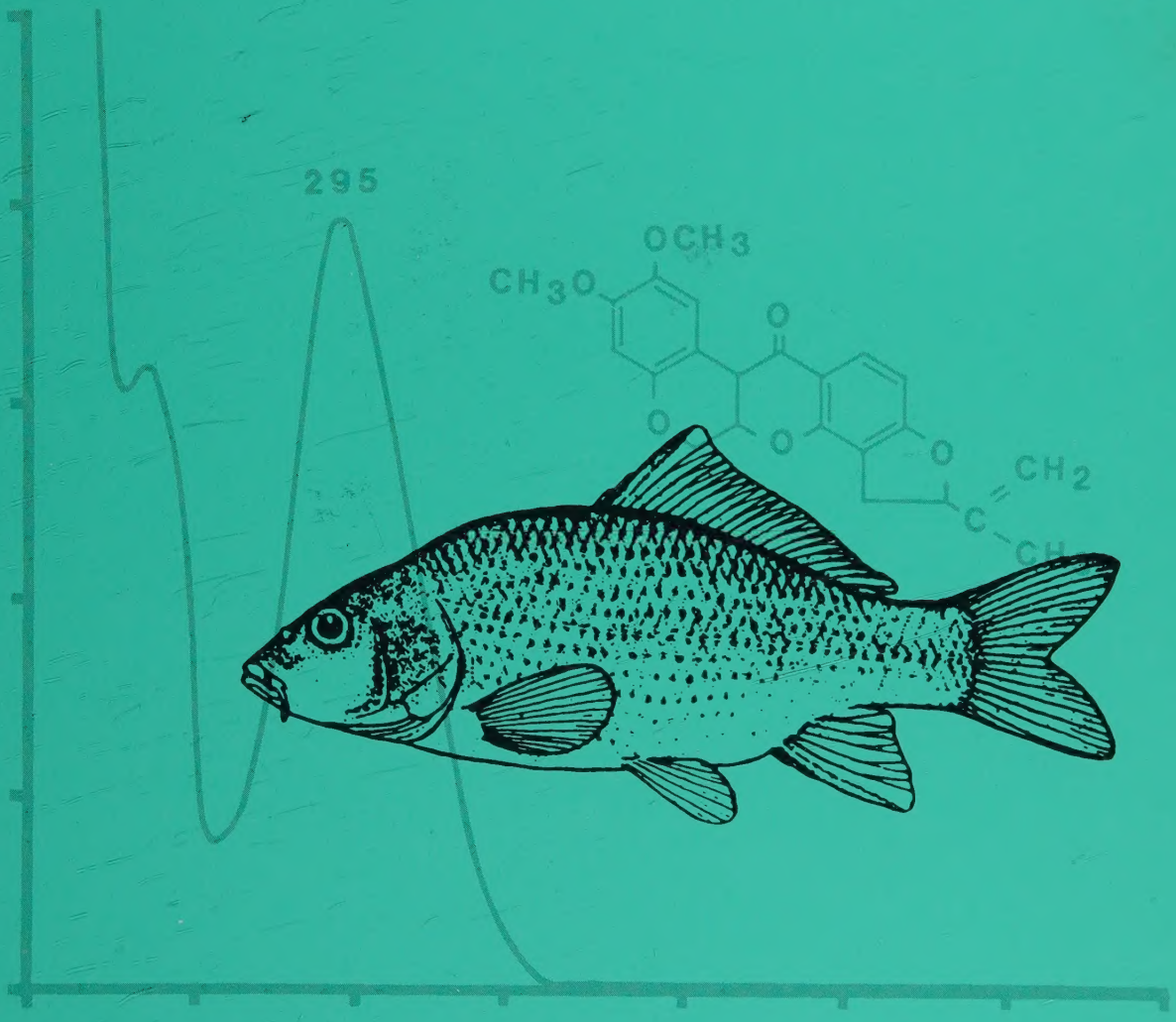
INVESTIGATIONS IN FISH CONTROL

95. Deposition and Persistence of Rotenone in Shallow Ponds During Cold and Warm Seasons



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Deposition and Persistence of Rotenone in Shallow Ponds During Cold and Warm Seasons

by

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Abstract

As part of the requirements for the continued registration of rotenone, data were developed on deposition and persistence of the chemical in aquatic ecosystems in cold and warm seasons. After treatment of two ponds, one in November (0–5° C) and one in July (23–27° C), with 5 μL/L of Noxfish (0.250 mg/L of rotenone), we collected samples of water, bottom sediments, invertebrates, and fish and analyzed them for rotenone by high performance liquid chromatography. Decomposition of rotenone in water followed a first-order decay curve; half-life was 10.3 days in cold water and 0.94 days in warm water. In cold water, residues in bottom sediments gradually increased to a peak of 0.1 μg/g after 14 days and then declined to <0.025 μg/g (the limit of detection) after 64 days; in warm water, residues in sediments fell below the detection limit within 24 h. In freshwater mussels and crayfish, residues gradually increased for 1 week in cold water and 1 day in warm water and then slowly decreased. Residues in fish varied with species and water temperatures; concentrations were higher in fish tissues than in the water on corresponding days.

Rotenone has been used as a fish toxicant in the United States for about 50 years, and is currently the most widely used piscicide in the United States (Schnick 1974). Since rotenone was first registered with the U.S. Environmental Protection Agency (EPA) in 1962, the requirements for registration of pesticides have increased and have been revised several times. The guidelines now require more extensive documentation of the safety, efficacy, and persistence of chemicals in the environment. All previously registered pesticides are required to conform to the revised standards when their labels are reviewed. Preliminary studies have indicated that the persistence of rotenone is brief in warm water and much longer in cold water (Gilderhus et al. 1986). Such information was helpful in developing adequate sampling schedules for more comprehensive studies.

We describe research performed in 1983 and 1984 on persistence of rotenone in the environment to meet current EPA requirements for continued registration. The objective of the studies was to determine where rotenone is deposited in the environment, in what amounts, and how long it stays there during different seasons. The studies were conducted in fall and summer in shallow ponds to determine the distribution and persistence of rotenone in water, bottom sediments, invertebrates, and fish under cold and warm conditions. For convenience, we refer to the pond treated in November as the "coldwater pond" and the one treated in July as the "warmwater pond." These designations do not imply any marked difference in thermal regimen or biota between the ponds.

Materials and Methods

Study Sites

The coldwater pond was at the Genoa (Wisconsin) National Fish Hatchery and the warmwater pond at the La Crosse National Fisheries Research Center; their physical, chemical, and sediment characteristics are shown in Table 1. Use of two different ponds was necessary because we did not have access to the hatchery pond

Table 1. *Characteristics of ponds used in studies on the persistence of rotenone in the environment.*

Characteristics	Coldwater pond	Warmwater pond
Pond and water		
Approximate dimensions (m)	137 × 33	27 × 8
Volume (m^3)	3,526	185
Surface area (ha)	0.46	0.02
Mean depth (m)	0.78	0.86
pH	8.62	8.35
Turbidity (NTU)[a]	3.43	2.65
Total hardness (mg/L as $CaCO_3$)	284	113
Total alkalinity (mg/L as $CaCO_3$)	253	92
Sediment		
Sand (%)	91	93
Silt (%)	7	4
Clay (%)	2	3
Organic content (%)	2.4	2.3
Moisture content (%)	41.1	29.0
Cation exchange capacity (meq/100 g)	14.45	13.04
Density (g/cm^3)	1.30	1.51
pH	8.2	8.1

[a] Nephelometric turbidity units.

during summer and the outlet structure in the pond at the Research Center would not withstand the pressure from thick ice. At the time of treatment of the coldwater pond on 14 November 1983, the water temperature was 5° C and remained at 4 to 5° C until 28 November, when the pond became covered with ice about 2.5 cm thick. Water temperature directly under the ice was 0° C by 5 December; ice thickness increased to 25 cm by 27 December and then decreased to 15 cm by 29 February. The warmwater pond was treated on 30 July 1984; water temperatures ranged from 23 to 27° C as long as 7 days after treatment, when rotenone was no longer detected in water or soil.

Treatments

Each pond was treated with Noxfish, a commercial formulation containing 5% rotenone, to produce a concentration of 0.250 mg/L of rotenone (the maximum allowed by the product label). The formulated chemical was diluted 8:1 with pond water before it was applied. In the coldwater pond, we used a boat with a venturi-type boat bailer on a 6-horsepower outboard motor (a small, shallow area at one end of the pond was treated with a hand-pumped sprayer). We treated the shallow end of the warmwater pond with the hand-pumped sprayer and the rest of the pond by using the boat bailer with the motor mounted on the water-control structure at the deep end of the pond.

Sampling

Samples of the various components in the aquatic environment (water, bottom soil, and experimental animals) were collected in accordance with Pesticide Assessment Guidelines, Subdivision N—Chemistry: Environmental Fate (EPA 1982). Sampling schedules were based on data from the preliminary studies on the persistence of rotenone in water (Gilderhus et al. 1986).

Water samples were collected in amber glass jugs submerged just below the surface of the water. Samples were collected along the midline of the coldwater pond at points 30, 60, and 90 m from one end, either from a boat or through holes in the ice made with an ice spud or gasoline-powered auger. Water samples from the warmwater pond were also collected along the midline, at points 3 m from either end and at the center of the pond. All water samples were extracted within 2 h after collection.

Samples of bottom soil were collected with a core sampler 5 cm in diameter, similar to that described by Swanson (1978), at the same locations where the water samples were collected. Each sediment sample was a composite of three cores taken within a radius of 0.5 m. Cores were collected by forcing the sampler 10–15 cm into the bottom, and then releasing the core into an enameled pan; the top 5 cm was then cut off and placed into a plastic container with a snap-on lid, along with the top 5 cm of each of the two other cores in the sample. Samples were placed in a freezer at −10° C within 2 h after collection.

Particle sizes of sediments were determined according to standardized procedures for sieving and hydrometer classification (ASTM 1979). Soil classifications were provided by the University of Wisconsin Soil and Forage Laboratory, Marshfield, Wisconsin.

Freshwater mussels and crayfish were used as representative invertebrates because they are easy to sample and are large enough to provide adequate tissue samples (10 g) for rotenone analysis. The mussels (*Lampsilis* sp.) were collected from Lake Onalaska near La Crosse, Wisconsin, and the crayfish (*Orconectes* sp.) were obtained from the Wisconsin Department of Natural Resources. Mussels were placed in a woven wire cage resting on the pond bottom, at a single location in each pond; sides of the cage extended above the water. A 10- to 15-cm layer of sand in the bottom of the cages provided a substrate in which the mussels could burrow. Mussels were placed in the coldwater pond 1 week before treatment and

the warmwater pond 2 weeks before treatment. At each sampling, six mussels were removed from the cage, rinsed in clean water for 30 s, and packaged as three replicates of two mussels each. The soft tissues were analyzed for rotenone and the shells were discarded.

Crayfish were placed in a single floating cage 1 week before treatment in each pond. At each sampling, three groups of three large crayfish were taken from the coldwater pond and three groups of six smaller crayfish from the warmwater pond (to ensure samples of adequate size for analysis). To aid handling and packaging, we killed crayfish immediately after collection by inserting a sharp blade into the brain. Whole crayfish were ground and extracted for rotenone analysis.

Invertebrates were wrapped in aluminum foil, placed in a polyethylene bag, and sealed. Samples were frozen within 2 h after collection and stored at $-10°$ C until analyzed.

Fish resident in the ponds (common carp, *Cyprinus carpio*, and largemouth bass, *Micropterus salmoides*, in the coldwater pond; shortnose gar, *Lepisosteus platostomus*, in the warmwater pond) were sampled after they were killed by rotenone on the day of treatment. Three common carp, three largemouth bass, and two shortnose gar were sampled and processed individually; the fillets and offal were packaged separately in foil, placed together in a plastic bag, and sealed.

Since resident fish killed by rotenone are not recommended for consumption as food, emphasis was placed on uptake and elimination of rotenone by fish that were stocked after the water was no longer toxic. Beginning on day 7 in the coldwater pond and day 1 in the warmwater pond, we placed fathead minnows, *Pimephales promelas*, in floating cages to monitor the toxicity of the water. When 9 of 10 fish survived for 24 h (on days 30 and 4 after treatment in coldwater and warmwater ponds, respectively) we considered the water to be safe for restocking. We chose to use an ictalurid and a centrarchid in each pond (different species of these families were avaliable to us when the two tests were conducted). Channel catfish, *Ictalurus punctatus*, and largemouth bass (mean total lengths, 37 and 23 cm, respectively) were placed in cages in the coldwater pond on day 30 after treatment. Black bullheads, *Ictalurus melas*, and bluegills, *Lepomis macrochirus*, averaging 25 and 13 cm in length, respectively, were placed in the warmwater pond on day 7 after treatment. Fish cages rested on the bottom and extended above the water surface. For the larger fish (channel catfish, black bullheads, and largemouth bass), three fish were taken at each sampling; the fillets and offal were packaged and analyzed separately for each fish. Bluegills were sampled as three groups of four fish on each sampling day; fillets and offal from each group of four were pooled and packaged separately. Packaging and freezing procedures for fish were the same as those used for invertebrates.

Sample Analyses

Water samples were acidified to pH 5 with an acetate buffer and extracted with a disposable Baker C_{18} chromatography column. The extracted rotenone was eluted from the column with 2 mL of methanol and analyzed by high performance liquid chromatography (HPLC) as described by Dawson et al. (1983). A Waters model M-45 HPLC with a Waters 15 cm $\times$ 3.9 mm Nova-Pak C_{18} reverse-phase column and UV detector (295 nm) was used for the analyses. The mobile phase consisted of methanol: water (70:30 v/v) at a flow rate of 1 mL/min. The lower limit of detection for water was 0.002 mg/L.

Sediment samples were extracted with methanol on a Sorval mixer, centrifuged, and filtered on Gelman type A/E glass fiber filters. The extracts were acidified, partitioned into hexane, and transferred to a silica gel column. The samples were eluted from the silica gel with benzene:acetone (97:3), exchanged into methanol, and analyzed by HPLC by the modified method of Bowman et al. (1978). The detection limit for rotenone in sediments was 0.025 μg/g.

Fish fillets, fish offal, crayfish (whole body), and freshwater mussels (without shell) were homogenized in a blender with dry ice according to the procedure of Benville and Tindle (1970). Homogenates were mixed with sodium sulfate and column-extracted with ethyl ether (Hesselberg and Johnson 1972). We separated extracts from lipids by gel permeation chromatography (GPC) with SX-3 biobeads and methylene chloride:cyclohexane (1:1 v/v). Further cleanup was obtained by silica gel chromatography in the same way as described for the sediment samples, and was followed by HPLC analysis. The lower limit of detection for rotenone in tissues was 0.005 μg/g. Quality assurance for the analytical portion of the study was provided by systematic analysis of blanks, replicates, and spiked controls.

Results

Water

Rotenone degraded much more slowly in the coldwater pond than in the warmwater pond (Table 2). In the cold-

water pond, the mean concentration of rotenone in the initial samples taken 3 h after treatment was 0.229 mg/L; the concentration declined gradually but steadily over time; and residues dropped to <0.002 mg/L (the limit of detection) after 57 days (Table 2). In the warmwater pond, the mean concentration of rotenone at 3 h was 0.180 mg/L; the concentration declined by more than 50% in the first 12 h; and residues fell to the 0.002-mg/L detection limit within 4 days (Table 2). The rate of loss of rotenone from water followed a first-order decay curve; the half-life was 10.3 days in the coldwater pond and 0.94 day (22.5 h) in the warmwater pond (Fig. 1).

Table 2. *Mean (N = 3) concentrations of rotenone (standard errors in parentheses) in samples collected from a coldwater and a warmwater pond treated with 5 μL/L of Noxfish (0.250 mg/L of rotenone).*

Pond and posttreatment time	Water (mg/L)	Sediments (μg/g)	Crayfish (μg/g)	Mussels (μg/g)
Coldwater[a]				
Hours				
3–6[b]	0.229	0.029	0.188	0.068
	(0.010)	(0.004)	(0.009)	(0.0000)
Days				
1	0.186	0.034	0.395	0.066
	(0.003)	(0.004)	(0.046)	(0.032)
3	0.164	0.058	0.340	0.174
	(0.004)	(0.008)	(0.014)	(0.048)
7	0.090	0.075	0.235	0.723
	(0.004)	(0.014)	(0.033)	(0.099)
14	0.062	0.100	0.200	0.696
	(0.003)	(0.025)	(0.065)	(0.033)
21	0.030	0.075	0.057	0.382
	(0.005)	(0.014)	(0.008)[c]	(0.145)
28	0.020	0.054	—	0.230
	(0.001)	(0.011)	—	(0.051)[c]
36	0.020	—	—	—
	(0.002)	—	—	—
43	0.012	0.042	—	—
	(0.000)	(0.008)	—	—
50	0.006	0.033	—	—
	(0.000)	(0.008)	—	—
57	0.002[d]	—	—	—
64	—	<0.025[d]	—	—
78	—	<0.025[d]	—	—
Warmwater[e]				
Hours				
3	0.180	—	—	—
	(0.002)	—	—	—
6	0.154	0.075	0.088	0.305
	(0.002)	(0.014)	(0.050)	(0.077)
12	0.110	0.033	—	—
	(0.005)	(0.008)	—	—
18	0.097	—	—	—
	(0.010)	—	—	—
Days				
1	0.089	<0.025[d]	0.076	1.060
	(0.001)	—	(0.00)	(0.427)[c]

Table 2. *Continued.*

Pond and posttreatment time	Water (mg/L)	Sediments (μg/g)	Crayfish (μg/g)	Mussels (μg/g)
1.5	0.061	—	—	—
	(0.001)	—	—	—
2	0.040	—	0.058	—
	(0.006)	—	(0.008)	—
3	0.020	—	0.045	—
	(0.000)	—	(0.022)	—
4	0.002[d]	—	0.019	—
	—	—	(0.017)	—
7	—	<0.025[d]	<0.005[e]	—

[a]Water temperature at time of treatment, 5° C.
[b]Water and sediments, 3 h; crayfish and mussels, 6 h.
[c]Last sample because no live animals remained at the next sampling date.
[d]Limit of detection.
[e]Water temperature at time of treatment, 24° C.

Sediments

Bottom sediments in the two ponds had similar physical characteristics (Table 1). Consequently, their adsorptive capacity for rotenone was probably similar because this capacity is closely related to particle size and organic content (Dawson et al. 1986). Residues of rotenone in bottom sediments in the coldwater pond peaked at 0.100 μg/g after 14 days and then declined to <0.025 μg/g (limit of detection) after 64 days (Table 2). Accumulation and elimination were much faster in the warmwater pond; the concentration in the sediments peaked at 0.075 μg/g after 6 h and dropped to <0.025 μg/g after 24 h (Table 2). The peak concentration in the sediments was higher than that in water in the coldwater pond but less than half that in water in the warmwater pond.

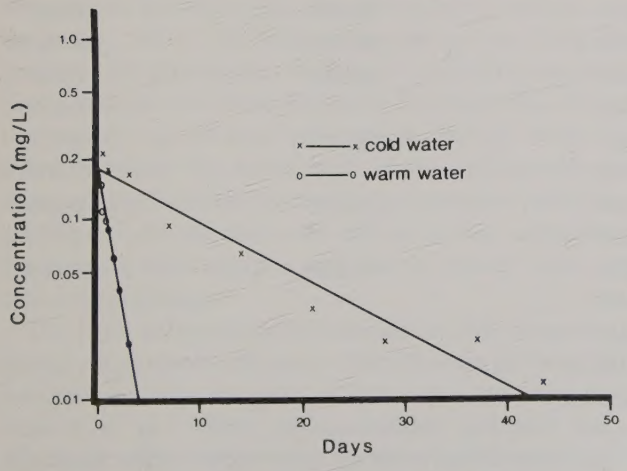

Fig. 1. Disappearance of rotenone from pond waters treated with 5 μL/L of Noxfish (0.250 mg/L of rotenone).

Crayfish

Crayfish in the coldwater pond accumulated concentrations of rotenone 1.58 times the treatment concentration by 1 day after treatment; concentrations then declined steadily from day 1 to day 21 (Table 2). All caged crayfish were dead by day 28, possibly as a result of the long submersion in cold water during a season when they would normally be hibernating. However, delayed mortality due to rotenone toxicity is also a possibility.

In warm water, residues of rotenone in crayfish peaked at 0.088 μg/g (35% of the treatment concentration) 6 h after treatment (Table 2). The concentration declined rapidly to the limit of detection (0.005 μg/g) by day 7. The decline of residues in crayfish closely paralleled that in water in the warmwater pond.

Mussels

The concentrations of rotenone accumulated were higher in mussels than in crayfish. In the coldwater pond, residues peaked at 0.723 μg/g (2.88 times the treatment concentration) at 7 days after treatment, and declined to 0.230 μg/g (Table 2) on day 28. In the warmwater pond, tissue concentrations reached 1.060 μg/g (4.24 times the treatment concentration) 1 day after treatment (Table 2). Because all of the mussels had died, no further sampling was possible. Since the caged mussels, which had been in the pond for 2 weeks, were alive on the day of treat-

ment and dead the next day, we assumed that the mortalities were due to the toxic effects of rotenone.

Fish

Fish in the ponds were dead within 2 to 4 h after treatment. Residues of rotenone in fillets from these fish were generally commensurate with the concentrations in the water. In the coldwater pond, common carp accumulated the highest concentrations (0.329 ± 0.046 μg/g in fillets) and largemouth bass the lowest (0.171 ± 0.008 μg/g in fillets). In shortnose gar in the warmwater pond, residues were 0.202 ± 0.116 μg/g in fillets. Concentrations in the offal were about double those in fillets of each species.

Fish stocked in the ponds after the rotenone had declined to nontoxic levels accumulated rotenone residues that were higher than those in the invertebrates that were in the pond on the day of treatment. In cold water, both channel catfish and largemouth bass accumulated rotenone residues in excess of 20 times the level in the water when the fish were stocked (Table 3). Peak concentrations in the fillets and offal of channel catfish were about equal (Table 3). In largemouth bass, the peak in the fillets was nearly 3 times that in the offal. In the warmwater pond, black bullheads concentrated rotenone up to 76 times the concentration in the water (0.002 mg/L) when the fish were stocked into the pond (Table 3). Their uptake and elimina-

Table 3. *Concentrations[a] of rotenone in channel catfish and largemouth bass stocked in a coldwater pond 30 days after it was treated with 5 μL/L of Noxfish (0.250 mg/L of rotenone) and in black bullheads and bluegills stocked in a warmwater pond 7 days after a similar treatment.*

Days after stocking	Channel catfish		Largemouth bass	
	Fillet	Offal	Fillet	Offal
1	0.123 (0.018)	0.228 (0.050)	0.082 (0.016)	0.166 (0.013)
3	0.360 (0.025)	0.351 (0.016)	1.225 (0.037)	0.440 (0.027)
6	0.410 (0.034)	0.417 (0.077)	0.502 (0.20)	0.263 (0.083)[b]
13	0.131 (0.011)	0.342 (0.068)	— —	— —
20	0.178 (0.026)	0.325 (0.038)[b]	— —	— —
	Black bullheads		Bluegills	
	Fillet	Offal	Fillet	Offal
1	0.005[c] (0.000)	0.032 (0.030)	0.064 (0.005)	0.067 (0.005)
3	0.005[c] (0.000)	0.054 (0.004)	<0.005[c] —	0.087 (0.008)
7	0.045 (0.042)	0.062 (0.003)	<0.005[c] —	0.078 (0.006)
14	0.153 (0.036)	0.096 (0.016)	<0.005[c] —	<0.005[c] —
21	0.083 (0.004)	0.026 (0.016)	<0.005[c] —	<0.005[c] —
29	0.060 (0.004)	0.018 (0.016)	— —	— —
35	<0.005[c] —	0.005[c] —	— —	— —

[a] Mean of three samples—standard errors in parentheses.
[b] Last sample because no live animals remained at the next sampling date.
[c] Limit of detection.

tion of rotenone spanned 5 weeks. In bluegills, the uptake and elimination of rotenone were rapid, peaking on day 1 and declining to below the limit of detection by day 3 (Table 3).

Discussion

The length of time that rotenone persisted in the water and the time that it became nontoxic to fathead minnows were about as expected. The degradation of rotenone in aquatic environments is influenced by many environmental factors that are more or less active, depending on the season. Water temperature was a major factor in our study, as in other published studies. The influence of temperature has been expressed in formulas developed by Post (1958) as well as by Engstrom-Heg and Colesante (1979), who also emphasized the effect of sunlight. Our data are in general agreement with those of Post (1958) and Engstrom-Heg and Colesante (1979). Post's formulas would have predicted persistence of about 46 days in our coldwater pond and 6 days in our warmwater pond. The formulas of Engstrom-Heg and Colesante (1979) would have predicted that the rotenone-treated water would remain toxic to fish somewhat longer (69 days in our coldwater pond and 7 days in our warmwater pond).

Various other factors are known to significantly affect the toxicity of rotenone (Gilderhus 1982). Aquatic plants and suspended clay presumably adsorb and absorb the chemical. Phytoplankton, zooplankton, and bacteria are also likely to influence the rate at which rotenone disappears from water and are likely to be most active in warm water. The generally higher concentrations of rotenone in sediments and organisms in the coldwater pond were probably due to the much longer time that they were exposed to detectable concentrations of rotenone in the water. All of the studies on rotenone persistence, including the present one, were based on limited data and thus cannot be considered definitive. To predict how long the toxicity of rotenone will persist, fishery workers should consider the entire body of relevant literature. Although published studies may help to estimate rotenone persistence, on-site tests with fish are always advisable for the final determination of when the treated water is safe for restocking.

The rapid accumulation of rotenone in fish tissues indicates a high uptake efficiency. The dynamics of chemical movement across the gills of fish were described by McKim et al. (1985), whose studies indicated that chemicals with octanol–water partition coefficients (log P) between 3 and 6 have the highest uptake in fish. Gingerich and Rach (1985) reported the log P for rotenone to be 4.26, placing it in the category of chemicals most easily transported across the gills. Those studies also showed that rotenone tended to concentrate in the viscera and that elimination from the viscera was relatively rapid. Rapid uptake of rotenone was also apparent in our study, in which the concentrations accumulated by fish were much higher than those in the water at the time the fish were stocked.

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As part of the requirements for the continued registration of rotenone, data were developed on deposition and persistence of the chemical in aquatic ecosystems in cold and warm seasons. After treatment of two ponds with 5 μL/L of Noxfish (0.250 mg/L of rotenone), we collected samples of water, bottom sediments, invertebrates, and fish and analyzed them for rotenone by high performance liquid chromatography. Decomposition of rotenone in water followed a first-order decay curve; half-life was 10.3 days in cold water and 0.94 days in warm water. In cold water, residues in bottom sediments gradually increased for 14 days and then declined to the limit of detection after 64 days; in warm water, residues in sediments fell below the detection limit within 24 h. In freshwater mussels and crayfish, residues gradually increased for 1 week in cold water and 1 day in warm water and then slowly decreased. Residues in fish varied with species and water temperatures; concentrations were higher in fish tissues than in the water on corresponding days.

Key words: Rotenone, residues, persistence, fish, water sediments, temperature effects.

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(Reports 87 through 89 are in one cover.)

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88. Influences of Selected Environmental Factors on the Activity of a Prospective Fish Toxicant, 2-(Digeranyl-amino)-ethanol, in Laboratory Tests, by C. A. Launer and T. D. Bills. 1979. 4 pp.
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